Determine Taz Activity Using RFP under PompC

In this experiment, varying concentrations of aspartate were added to a culture of *E. coli* expressing Taz under Pcat and RFP under the promoter PompC. The experiment was based on Dundee iGEM 2013 team's experiment with ompC-GFP reporter construct (BBa_K1012005 designed by John Allan) (Harrison, 2013) and the research of Michalodimitrakis, Sourjik and Serrano who tested Taz expression using GFP under PompC regulation (Michalodimitrakis, Sourjik, & Serrano, 2005)

Experiment Description:

Objectives:

- Determine activity level of Tar-EnvZ chimaera protein.
- Characterize the EnvZ/ompR system by using varying concentrations of aspartate and observing RFP expression.

Materials and Equipment:

- *E. coli* :
  - Top10 containing RFP under Plac
  - Top10 (blank)

  Isogenic strains containing mRFP under PompC (pSB1C3) and Taz construct (pSB1AK3):
  - BW21153
  - JW3367-3 – ΔenvZ

  Isogenic strains (for blank):
  - BW21153
  - JW3367-3 – ΔenvZ (Kan resistance)
- 50ml Falcons
- LB medium
- Antibiotics (CM, Amp and Kan)
- Bioassay medium
- 4x 500ml Erlenmeyer flasks (sterile)
- 3x 250ml Erlenmeyer flasks (sterile)
- 48 well plate (and cover) **– label A-F and 1-8**
- L-aspartic acid solution from Sigma-Aldrich 38mM
- several 96 well plates for plate reader
- Plate reader
- Multi-Pippetor, Pippettes, pippettors, tips

Method:

1. Grow the cells in LB medium + appropriate antibiotics at 37°C overnight (in a falcon)
2. **Tester strains**: transfer 1ml aliquot to 100ml LB containing appropriate antibiotics (in 500ml Erlenmeyer flask)
   Blank strains: transfer 5ml aliquot to 50ml LB containing appropriate antibiotics (in 250ml Erlenmeyer)
3. Grow at 37°C until OD_{600} of 0.6.
4. Transfer **all the remaining culture** to 50ml Falcon tubes, mark the volume on the falcon itself.
5. Pellet cells (centrifuge 10 min at 4000rpm) and then pour off the supernatant.
6. Vortex to break the pellet.
7. Add bioassay medium to falcons up to the volume marked. Re-suspend the cells in this bioassay.
8. Prepare the 48 plate as follows (see Figure 1 and Table 1 below) (use multipipettor):
   a. Add 2.6ml culture (bioassay + cells) to the appropriate sample wells in column A.
   b. Add 2ml culture (bioassay + cells) to the appropriate sample wells in column B-F.
   c. Add 300µl 38mM L-aspartate solution to the sample wells in column A (get concentration of ~4mM)
   d. Perform x4 dilutions as follows:
      Transfer 667µl from sample wells in column A to column B. Transfer 667µl from sample wells in column B to column C. Continue in this fashion until column F.

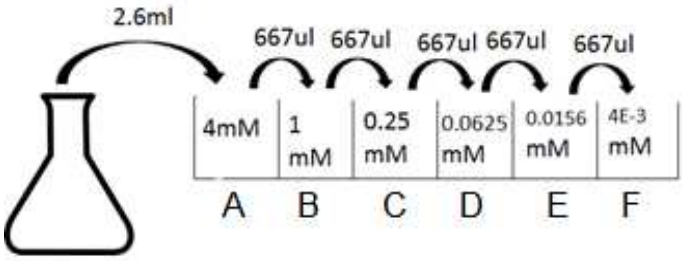

Figure 1: dilution diagram

Blank preparation:
Add 2ml bioassay+cells (without plasmids) to the "blank" wells.

9. After 3 hours remove 200µl from each well and transfer to the plate-reader plate and read absorbance (OD600 – for cell concentration) and fluorescence (excitation peak: 584nm, emission peak: 608nm) with plate reader. Repeat every 30 minutes for 2 hours. Use each 96 well plate for two readings. Use half for one reading and the other half for another reading. (see table 2)
10. Calculate relative expression on graph:

$$Expression = \frac{F}{OD_{600}} - \left(\frac{F}{OD_{600}}\right)_{blank}$$

References

Harrison, K. (2013). *Reporter ompC-GFP*. Retrieved from Toximop: http://2013.igem.org/Team:Dundee/Project/ReporterOmpC

Michalodimitrakis, K. M., Sourjik, V., & Serrano, L. (2005). Plasticity in amino acid sensing of the chimeric receptor Taz. *Molecular Microbiology*, *58*(1), 257–266.

Table 1: 48 well plate reader

	A	B	C	D	E	F
1	BW+4mM asp	BW+1mM asp	BW+0.25mM asp	BW+0.0625mM asp	BW+0.0156mM asp	BW+4E-3mM asp
2	BW+4mM asp	BW+1mM asp	BW+0.25mM asp	BW+0.0625mM asp	BW+0.0156mM asp	BW+4E-3mM asp
3	JW+4mM asp	JW+1mM asp	JW+0.25mM asp	JW+0.0625mM asp	JW+0.0156mM asp	JW+4E-3mM asp
4	JW+4mM asp	JW+1mM asp	JW+0.25mM asp	JW+0.0625mM asp	JW+0.0156mM asp	JW+4E-3mM asp
5	Top10+4mM asp	Top10+1mM asp	Top10+0.25mM asp	Top10+0.0625mM asp	Top10+0.0156mM asp	Top10+4E-3mM asp
6	Top10+4mM asp	Top10+1mM asp	Top10+0.25mM asp	Top10+0.0625mM asp	Top10+0.0156mM asp	Top10+4E-3mM asp
7	**Blank BW**	**Blank JW**	**Blank Top10**	*BW+0mM asp*	*JW+0mM asp*	*Top10+0mM asp*
8	**Blank BW**	**Blank JW**	**Blank Top10**	*BW+0mM asp*	*JW+0mM asp*	*Top10+0mM asp*

Table 2: 96 well plate for x3 dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW+4mM asp	BW+1mM asp	BW+0.25mM asp	BW+0.0625mM asp	BW+0.0156mM asp	BW+4E-3mM asp	BW+4mM asp	BW+1mM asp	BW+0.25mM asp	BW+0.0625mM asp	BW+0.0156mM asp	BW+4E-3mM asp
B	BW+4mM asp	BW+1mM asp	BW+0.25mM asp	BW+0.0625mM asp	BW+0.0156mM asp	BW+4E-3mM asp	BW+4mM asp	BW+1mM asp	BW+0.25mM asp	BW+0.0625mM asp	BW+0.0156mM asp	BW+4E-3mM asp
C	JW+4mM asp	JW+1mM asp	JW+0.25mM asp	JW+0.0625mM asp	JW+0.0156mM asp	JW+4E-3mM asp	JW+4mM asp	JW+1mM asp	JW+0.25mM asp	JW+0.0625mM asp	JW+0.0156mM asp	JW+4E-3mM asp
D	JW+4mM asp	JW+1mM asp	JW+0.25mM asp	JW+0.0625mM asp	JW+0.0156mM asp	JW+4E-3mM asp	JW+4mM asp	JW+1mM asp	JW+0.25mM asp	JW+0.0625mM asp	JW+0.0156mM asp	JW+4E-3mM asp
E	Top10+4mM asp	Top10+1mM asp	Top10+0.25mM asp	Top10+0.0625mM asp	Top10+0.0156mM asp	Top10+4E-3mM asp	Top10+4mM asp	Top10+1mM asp	Top10+0.25mM asp	Top10+0.0625mM asp	Top10+0.0156mM asp	Top10+4E-3mM asp
F	Top10+4mM asp	Top10+1mM asp	Top10+0.25mM asp	Top10+0.0625mM asp	Top10+0.0156mM asp	Top10+4E-3mM asp	Top10+4mM asp	Top10+1mM asp	Top10+0.25mM asp	Top10+0.0625mM asp	Top10+0.0156mM asp	Top10+4E-3mM asp
G	**Blank BW**	**Blank JW**	**Blank Top10**	*BW+0mM asp*	*JW+0mM asp*	*Top10+0mM asp*	**Blank BW**	**Blank JW**	**Blank Top10**	*BW+0mM asp*	*JW+0mM asp*	*Top10+0mM asp*
H	**Blank BW**	**Blank JW**	**Blank Top10**	*BW+0mM asp*	*JW+0mM asp*	*Top10+0mM asp*	**Blank BW**	**Blank JW**	**Blank Top10**	*BW+0mM asp*	*JW+0mM asp*	*Top10+0mM asp*